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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER	
STOICA, ELLY GERALD	

ART UNIT	PAPER NUMBER
1647	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/522,826	MENART ET AL.	
	Examiner	Art Unit	
	ELLY-GERALD STOICA	1647	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *03 October 2008*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,3-7,10,12-17,19-21,23-26,38 and 39* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1, 3-7, 10, 12-17, 19-21, 23-26, 38-39* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 10/03/2008 has been entered. Claims 1, 3-7, 10, 12-17, 19-21, 23-26, 38-39 are pending and under examination. Claims 2, 8, 9, 11, 18, 22, 27-37 are cancelled.

Claim Rejections - 35 USC § 112

2. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

3. Claims 1, 3-7, 10, 12-17, 19-21, 23-26, 38-39 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Specifically, in the independent claim 1 it is unclear if the recitation "cultivated organism having one or more cells" defines a unicellular or multicellular organism and what is the nature of this organism. Thus, the metes and bounds of the claims could not be determined.

The term "substantially" in claims 1 and 26 is a relative term which renders the claims indefinite. The term "substantially" as a qualifier to the "correctly folded protein"

is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. Therefore, the metes and bounds of the claims could not be determined.

Where applicant acts as his or her own lexicographer to specifically define a term of a claim contrary to its ordinary meaning, the written description must clearly redefine the claim term and set forth the uncommon definition so as to put one reasonably skilled in the art on notice that the applicant intended to so redefine that claim term. *Process Control Corp. v. HydReclaim Corp.*, 190 F.3d 1350, 1357, 52 USPQ2d 1029, 1033 (Fed. Cir. 1999). The term "inclusion bodies having an aqueous solubility in the cells of the organism" in claim 1 is used by the claim to mean "non-classical inclusion body", while the accepted meaning does not exist per se, being a contradiction in terms. By their nature, inclusion bodies are particulate and insoluble in the cells. The term is indefinite because the specification does not clearly redefine the term; if the term is meant to refer to "non-classical inclusion bodies" which are more soluble (in media under non-denaturating conditions such as non-denaturating aqueous solutions) than it should be claimed as such. Otherwise, the metes and bounds of the claim could not be determined.

Claim 1 is further indefinite because it is unclear how the "principle of performing the fermentation" can be regulated so as to perform the method step claimed. As such, the metes and bounds of the claim cannot be determined.

Also the metes and bounds of "an agent **capable** of causing stress" could not be determined since the agent is just capable of doing it but is not known if it actually is causing stress.

In addition, claims 3, 12-15, 26 are indefinite because they contain series of abbreviations which were not adequately spelled out in the claims or specification; as such the metes and bound of the claims cannot be determined.

Claims, 3-7, 10, 12-17, 19-21, 23-26, 38-39 are rejected as dependent claims.

4. Claims 1, 3, 5-7, 10, 12-17, 19-21, 23-26, 38-39 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for production of G-CSF, does not reasonably provide enablement for any heterologously expressed protein. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims.

The factors considered when determining if the disclosure satisfies the enablement requirement and whether any necessary experimentation is "undue" include, but are not limited to: 1) nature of the invention, 2) state of the prior art, 3) relative skill of those in the art, 4) level of predictability in the art, 5) existence of working examples, 6) breadth of claims, 7) amount of direction or guidance by the inventor, and 8) quantity of experimentation needed to make or use the invention. In re Wands, 858 F.2d 731,737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988).

The claims are drawn to a process for the production of a biologically active protein, comprising:

expressing said protein as a heterologous protein in an expression system comprising a cultivated organism having one or more cells, wherein the protein is expressed as a substantially correctly folded protein precursor in inclusion bodies having an aqueous solubility in the cells of the organism;

regulating one or more cultivation parameters selected from the group consisting of temperature of cultivation, composition of cultivation medium, induction mode, principle of performing the fermentation, addition of an agent capable of causing stress, and co-expression of auxiliary proteins, wherein regulating the one or more parameters increases the proportion of substantially correctly folded protein precursor present in the inclusion bodies in the cells, relative to the proportion of substantially correctly folded protein precursor present in inclusion bodies in cells of an organism not cultivated by regulating said parameters;

isolating the inclusion bodies from the cells of the .organism; optionally, washing the inclusion bodies;

solubilizing the substantially correctly folded protein precursor from the inclusion bodies under non-denaturing conditions;

and purifying the biologically active protein from the solubilized substantially correctly folded protein precursor.

The claims also present specific limitations with regard to the specific bacterium used, cultivation conditions (temperature, growth media, auxiliary proteins) or solubilizing conditions.

The prior art is aware of certain growing conditions which lead to inclusion bodies that are capable of being solubilized without being exposed to denaturating agents (i.e., inclusion bodies containing correctly folded proteins) for specific polypeptides (Gonzales-Villasenor, L.I., U.S. Pub. 20030166062; Li et al. U.S. Pat. 5,912,327; Patra et al., Protein Expression and Purification, 18, 182-192, 2000). However, the examples are targeted to specific proteins and also the art mentions that the process is still not considered routine and individual proteins have different conditions to be determined by trial an and error experiments, especially for big proteins with multiple disulfide bonds in their correctly folded structure (Georgiou et al., Current Opinion in Biotechnology 7, 190-197, 1996- Introduction and Conclusion sections)..

The specification presents detailed guidance and working examples for G-CSF only. However the breadth of the claims is directed to any biologically active protein without any indication of certain particular structures. Applying the general directions as exemplified for G-CSF would not put the skilled artisan in possession of the invention since the process and the product cannot be judged as successful until experimentation is performed. As such, for other proteins that G-CSF, the specification is just a hypothetical process that is uncertain in its feasibility and a huge amount of experimentation is needed to validate it for every biologically active protein.

Due to the large quantity of experimentation necessary to generate the vast number of biologically active proteins recited in the claims and possibly screen the same for activity; the lack of direction/guidance presented in the specification regarding which structural features are required in order to assure the production of any other protein than G-CSF; the absence of working examples directed to other proteins than G-CSF; the state of the prior art which establishes the unpredictability of obtaining correctly folded proteins in the inclusion bodies in any conditions; and the breadth of the claims which fail to recite any structural or functional limitations, undue experimentation would be required of the skilled artisan to use the claimed invention in its full scope.

Claim Rejections - 35 USC § 102

5. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.
>
> (e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

6. Claims 1, 5, 6, 10, 12-17, 23-25 are rejected under 35 U.S.C. 102(e) as being anticipated by Gonzales-Villasenor, LI (U.S. Pub. No.20030166062, filed 02/22/2002).

Gonzales-Villasenor teaches a method for solubilizing and recovering, in bioactive and isolated form with retained native state configuration, a target peptide from a host organism in which the heterologous polypeptide is present in insoluble form. Broadly this method comprises (i) disrupting the host cell to produce a lysate (ii) recovering lysate precipitate containing the polypeptide (iii) resuspending the lysate precipitate in a denaturant-free, non-buffered solubilization solution to produce a solubilization preparation that comprises both sodium hydroxide between about 8 and about 10 mM and the target peptide between about 1 and about 4 mg peptide per ml solubilization solution, wherein the resultant solubilization preparation has a pH of between about 9 and about 11.2; (iv) recovering supernatant from the solubilization preparation containing non-denatured target peptide (abstract). The method is used to solubilize recombinant polypeptides from inclusion bodies produced by fermentation in the bacteria, Escherichia coli, and to stabilize the solubilized recombinant proteins to preserve their biological activity. the solubilization of inclusion bodies is carried out in an aqueous solution called the `solubilization solution` at elevated pH, preferably NaOH between about 8 and about 10 mM and pH of between about 10.5 to about 11.0, and at low pH, preferably with HCL between about 10 to about 20 mM and pH between about 2.2 to about 2.6 at protein concentrations of between about 2 to about 10 mg/ml depending on the pH. The solubilization solution includes stabilizers preferably Mannitol between about 2 and about 2.5 mM and Lactose between about 1 and about 2 mM. The time of solubilization of inclusion bodies is dependent on the overall charge of the protein, pH, compounds in the solvent, and temperature, and is easily determined

empirically for each different polypeptide following the procedure. Stabilization of crude and isolated biologically active recombinant proteins is carried out by dialysis of about 48 hours or by ultrafiltration/diafiltration into an aqueous solution named the "stabilization solution" containing about 30 to about 40 mM sodium bicarbonate pH about 8.0 or about 10 to 20 mM sodium phosphate pH about 8.0 and about 5 to about 10 mM lactose. The methods are used as methods for the solubilization and stabilization of recombinant proteins that are sequestered in inclusion bodies that have been obtained by fermentation in a microbial host such as bacteria or yeast (Escherichia coli and Saccharomyces cerevisiae) and are applied to monomeric proteins in the range of about 16 to about 60 KDa with high and low content of hydrophobic amino acid residues, a high level of positively and/or negatively charged amino acid residues and several cysteine residues such as fish somatotropin and prolactin, and human fast twitch skeletal muscle Troponin I ([0031]-[0045]). The E. Coli is grown at lower temperatures (30° C) ((Example 6). Transfected host organisms are grown under conditions permissive for protein expression. Induction includes temperature modulation or addition of IPTG. Fermentation is carried out under conditions of sufficient time, temperature, and pH, to result in the formation of inclusion bodies comprising the recombinant protein within host cells ([0141]). The IPTG concentration used is 0.4mM (Example 12). The aqueous solubilization buffer is used at concentrations between 5 and 40mM and may include phosphate buffer ([0046] and [0091]).

Thus, the teachings of Gonzales-Villasenor anticipate the claims 1, 5, 6, 10, 12-17, 23-25.

7. Claims 1, 5, 6, and 12-14 are rejected under 35 U.S.C. 102(b) as being anticipated by Li et al. (U.S. Pat. 5,912, 327).

Li et al. teach methods of obtaining solubilized, biologically active target protein of higher yield and better purity than the processes known in the prior art which use 6-8 M Guanidinium Chloride for solubilization of inclusion bodies from bacterially expressed proteins. Inclusion bodies are released from cells by lysis, optionally washed to remove cellular components prior to extraction, and extracted with solutions containing low concentrations of guanidine salts. The methods are used for solubilizing inclusion bodies by treatment with guanidine salts at concentrations of about 0.7 to about 3.5M. Further, the proteins can be refolded by rapid dilution of guanidine salt extracts and optionally employ agents which facilitate target protein refolding and further purification by chromatographic methods (col. 2, lines 5-40). The proteins were obtained in inclusion bodies in E. Coli induced with IPTG at 1mM (Example 1).

Thus, claims 1, 5, 6 and 12-14 are anticipated by Li et al.

8. Claims 1, 5-7, 12-14, 16, 17 and 23-26 are rejected under 35 U.S.C. 102(b) as being anticipated by Patra et al. (Protein Expression and Purification 18,182–192, 2000).

Patra et al teach methods of obtaining recombinant human growth hormone (r-hGH) from E. Coli inclusion bodies. The Inclusion bodies from the cells were isolated and purified to homogeneity. Various buffers with and without reducing agents were

used to solubilize rhGH from the inclusion bodies. Complete solubilization of the inclusion bodies was obtained with Tris/HCl buffer containing 2M urea. Biologically active GH was obtained after further purification by ion-exchange chromatography. The E. Coli in which the heterologous protein was expressed was grown in a 3.5 l fermentor in a fed-batch mode with a continuous supply of glucose and yeast extract (at 10g/L and the culture was induced with 1 mM IPTG. The inclusion bodies were washed with Tris-HCl buffer and with water. The solubilizing of the hGH from the inclusion bodies was performed with 2M Tris buffers at pH about 12 with or without 2M Urea (Material and Methods). The level of expression of r-hGH was around 13% of the total cellular protein (Results, first full paragraph).

Thus the teachings of Patra et al. anticipate claims 1, 5-7, 12-14, 16, 17 and 23-26 of the instant Application.

9. Claims 1, 5, 6, 12-14, 16, 17, 23 and 25 are rejected under 35 U.S.C. 102(b) as being anticipated by Panda et al. (J. Biotechnology, 75, 161-172, 1999).

Panda et al. teach a process for maximizing the volumetric productivity of recombinant ovine growth hormone (r-oGH) expressed in Escherichia coli during high cell density fermentation process. Kinetics of r-oGH expression as inclusion bodies and its effect on specific growth rates of E. coli cells were monitored during batch fermentation process. It was observed that during r-oGH expression in E. coli, the specific growth rate of the culture became an intrinsic property of the cells which is reduced in a programmed manner upon induction. Nutrient feeding during protein expression phase of the fed-batch process was designed according to the reduction in

specific growth rate of the culture. By feeding yeast extract along with glucose during fed-batch operation, high cell growth with very little accumulation of acetic acid was observed. Use of yeast extract helped in maintaining high specific cellular protein yield which resulted in high volumetric productivity of r-oGH (abstract). The cultures were added IPTG at 1mM concentration in various stages of the fermentation process (Table 2). The inclusion bodies formed were separated from the E. Coli cells and washed with water and contained monomeric recombinant ovine Growth Hormone in proportion of 95%. They were solubilized in Tris-HCl buffer containing a non-denaturating concentration (1%) of Sodium Dodecyl Sulfate (Material and Methods).

Thus, the teachings of Panda et al. anticipate claims 1, 5, 6, 12-14, 16, 17, 23 and 25 of the instant Application.

Claim Rejections - 35 USC § 103

10. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

11. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was

not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

12. The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

13. Claims 3, 4, 19-20, 26, 38 and 39 are rejected under 35 U.S.C. 103(a) as being unpatentable over Panda et al. (J. Biotechnology, 75, 161-172,1999) in view of Souza LM (U.S. Pat. No. 4,810,643) and in further view of Ambrosius et al. (U.S. Pat. No. 5,618,927), Camble et al. (U. S. Pat. 5,773,581), and Pelleymounter et al. (U.S. Pub. 20020009798).

The independent claim1 is drawn to a process for the production of a biologically active protein, comprising:

> expressing said protein as a heterologous protein in an expression system comprising a cultivated organism having one or more cells, wherein the protein is expressed as a substantially correctly folded protein precursor in inclusion bodies having an aqueous solubility in the cells of the organism;
>
> regulating one or more cultivation parameters selected from the group consisting of temperature of cultivation, composition of cultivation medium, induction mode,

principle of performing the fermentation, addition of an agent capable of causing stress, and co-expression of auxiliary proteins, wherein regulating the one or more parameters increases the proportion of substantially correctly folded protein precursor present in the inclusion bodies in the cells, relative to the proportion of substantially correctly folded protein precursor present in inclusion bodies in cells of an organism not cultivated by regulating said parameters;

isolating the inclusion bodies from the cells of the .organism; optionally, washing the inclusion bodies;

solubilizing the substantially correctly folded protein precursor from the inclusion bodies under non-denaturing conditions;

and purifying the biologically active protein from the solubilized substantially correctly folded protein precursor.

The limitations that the claims 3 and 4 add are that the protein obtained is G-CSF.

The limitations that the claims 19-20, 26, 38 and 39 add consist of specific fermentation media (GYST, GYSP, LYSP LYST, LBON or GYSPON) and the use of non-denaturating solution of N-Lauroyl sarcosine in ranges of about 0.1% to about 0.25% mass per volume and a high solubilizing concentration of a buffer which may be HEPES.

The teachings of Panda et al. were presented supra. Panda et al. are silent about the use of the specific cultivation media cited *supra*, about the use of N-Lauryl sarcosine

for solubilizing the inclusion bodies or about obtaining recombinant G-CSF from the inclusion bodies formed in E. Coli.

Panda et al. present, in table 1, the composition of their medium. The composition is not the same as the GYST, GYSP, LYSP LYST, LBON or GYSPON. In the specification of the instant Application the composition of the media is described ([0074]). One of the buffers (GYST) differs from the classic E. Coli growth medium LB by containing 10g/l glucose and metals in traces. The medium taught by Panda et al. contains 10g/l glucose and metal in traces. This shows that it was routine in the art to adapt the composition for the bacterial growth media to accommodate various growth conditions.

Souza et al. teach E. Coli expression of human recombinant G-CSF by cultivation of the cells in LB broth and by modulating the temperature (col. 16, lines 1-15). The density of growth is low though and the cells contained between 3-5% G-CSF.

Ambrosius et al. teach solubilization and renaturation steps are necessary in order to convert proteins are produced in prokaryotic cells such as E. coli, (in inclusion bodies) into their active form. The process according to the present invention can be carried out in one of two ways. One variant is to work with a Tris buffer of the stated concentration so that Tris or/and a Tris salt is also used for adjusting the pH. The second variant is to work with a buffer which has previously been described for the corresponding process and to additionally add Tris or/and a salt of Tris. This means that the pH value of the incubation solution is adjusted by a buffer substance which is different from Tris. In both cases it is expedient to take care that the addition of Tris or

the increase in Tris concentration does not result in a change in pH. The process according to the present invention comprises the incubation of the inclusion bodies with a Tris solution which has a concentration of between 400mM to 2M. Examples of proteins to be treated include G-CSF. The advantages of the reactivation process according is an increase in the final yield of active protein of 30 to 300% compared to a process in which a buffer is used at a lower concentration (col. 2, line 47 to col.3, line 36).

Camble et al. teaches producing G-CSF from an inclusion body by suspending said inclusion body in a detergent, particularly N-lauroyl sarcosine in salt form (e.g. Sarkosyl) at concentrations well bellow 1%, and as low as 0.2%, which will make the final removal of the detergent for obtaining an active protein easier (col.10, lines 12-55).

Pelleymounter et al. teach the use of HEPES buffer together with N-lauroyl sarcosine for processing inclusion bodies containing murine OB protein.

It would have been obvious for a person of ordinary skill in the art at the time that the invention was made to combine the teachings of Panda et al. and Souza et al. to obtain a high yield of G-CSF with a reasonable expectation of success, since Panda et al. improved the growth condition for obtaining proteins in inclusion bodies and Souza et al. describe a lower yield for using just the typical LB broth. Further, in processing the inclusion bodies it would have been obvious to process the inclusion bodies according to the combined teachings of Ambrosius et al., Camble et al. and Pelleymounter et al. with a reasonable expectation of success since each of them added improvement over the classical processes of denaturation-renaturation proteins from inclusion bodies. A

person of ordinary skill in the art is always motivated to pursue the known options within her or his technical grasp. If this leads to the anticipated success, it is likely the product not of innovation but of ordinary skill and common sense.

14. Claim 21 is rejected under 35 U.S.C. 103(a) as being unpatentable over Patra et al. (Protein Expression and Purification 18,182–192, 2000) in view of Donnelly et al. (U.S. Pat. No. 6,677,139).

The claim adds to the independent claim 1 (presented supra) the limitation that the stressing agent is selected from ethanol or propanol.

The teachings of Patra et al. were presented supra. They are silent about the use of ethanol or propanol in the growth media for E. Coli.

Donnelly et al. teach methods for the production of proteins bacterial cells, methods which use a fusion protein comprising a chaperonin binding domain in host cells induced or regulated to have increased levels of chaperonin which binds the chaperonin binding domain (Abstract). Specifically, the effect of the GroES-loop leader sequence on expression of BAX protein was evaluated in cultures that enhance the expression of E. coli chaperones in the cell. The rationale was based on the assumption that folding of expressed fusion proteins would be mediated through interaction with the chaperonin GroEL. When grown in the presence of moderate concentrations of ethanol, E. coli is known to induce higher levels of chaperones and other stress proteins (example3).

It would have been obvious for a person of ordinary skill in the art at the time that the invention was made to have modified the teachings of Patra et al. with the teachings

of Donnelly et al. with a reasonable expectation of success since the method of Donnelly was used for obtaining even a toxic protein (for E. Coli) which is correctly folded due to the chaperone protein effect. A person of ordinary skill in the art is always motivated to pursue the known options within her or his technical grasp. If this leads to the anticipated success, it is likely the product not of innovation but of ordinary skill and common sense.

15. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure:

- Waldo et al., (Nature Biotechnology 17, 691-695, 1999) teach the temperature dependence of the misfolding of proteins produced in E. Coli (p. 692, right col., first full paragraph).
- Thomas et al. (Protein Expression and purification, 11, 289-296, 1997) teach that proper folding of aggregation-prone recombinant proteins in E. Coli is facilitated by co-overexpression of molecular chaperones or by culturing the cells in the presence of ethanol that regulates the synthesis of all heat shock proteins (abstract).

Conclusion

16. No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ELLY-GERALD STOICA whose telephone number is (571)272-9941. The examiner can normally be reached on 8:30-17:00 M-F.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Manjunath N. Rao can be reached on (571) 272-0939. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Christine J Saoud/
Primary Examiner, Art Unit 1647